EFFECT OF ZINC IONS ON THE EXCITABLE MEMBRANE OF THE SKELETAL MUSCLE FIBER

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Zinc (Zn^{++}) ions are constituents of many metalloenzymes. The psoas muscle of the rabbit is known to contain zinc in a concentration comparable with its calcium concentration [5]. Little is known of the function of zinc in the contractile act of the living muscle, although it has an appreciable influence on its mechanical activity. It has been shown, for example, that by the action of Zn^{++} ions on the sartorius muscle of the frog the tension is increased 2-3 times, the periods of contraction and relaxation are lengthened, and the length of the refractory period is increased 4 times [7].

Intracellular recordings of the membrane potential of a muscle have shown [6] that zinc ions, in a concentration of $5 \cdot 10^{-5}$ moles, double the duration of the action potential (AP) as a result of an increase in the duration of its descending phase. The blocking action of Zn^{++} in a concentration of 0.5 mmole on transmission in the neuromuscular synapse has also been demonstrated [12].

The object of the present investigation was to study the action of Zn^{++} ions on the properties of the membrane of the muscle fiber.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of the frog (Rana temporaria). The muscle was fixed in a special bath in a slightly stretched state. Intracellular recordings of the electrical activity of the muscle were made by means of glass microelectrodes. The resistance of the microelectrodes was 10-40 mΩ. Two separate microelectrodes were introduced into one muscle fiber. One electrode was used to pick up the potential, the other to stimulate the fiber (Fig. 1). The potential difference on the membrane was measured by means of a two-channel dc amplifier and a cathode repeater, assembled in accordance with the scheme of A. L. Byzov and M. M. Bongard [1]. The resting potential, the action potential, and the stimulating current were measured by comparing them with a known calibration potential.

To measure the input resistance of the membrane of the muscle fibers rectangular pulses of hyperpolarizing current were used. The solutions used in the experiment were supplied from special vessels into the working chamber in which the muscle was placed. Solutions of $ZnCl_2$ were used in the experiments in concentrations of 0.05-1.0 mmole. The investigation lasted 1 year. The composition of the Ringer's solution was (per liter of distilled water): 6.5 g NaCl, 140 mg KCl, 120 mg $CaCl_2$, 200 mg $NaHCO_3$ (in the autumn-winter period), and 6.5 g NaCl, 100 mg KCl, 200 mg $CaCl_2$, 200 mg $NaHCO_3$ (in the spring-summer period).

EXPERIMENTAL RESULTS AND DISCUSSION

In Ringer's solution for cold-blooded animals the resting potential (RP) was 81.2 ±1.7 mV. When the Ringer's solution was replaced by a solution containing $ZnCl_2$ in a concentration of 0.05-0.1 mmole, the RP was 81.3 ±2.4 mV

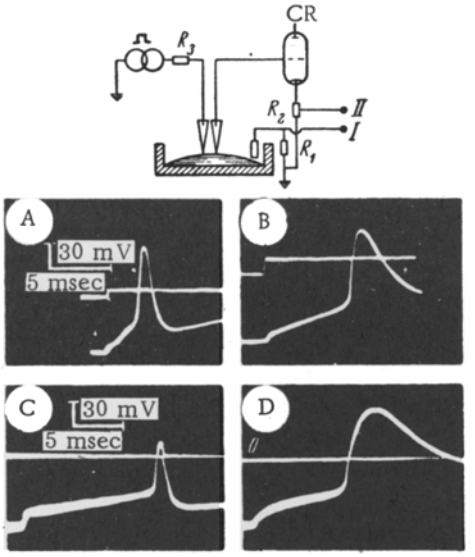

Fig. 1. Action of Zn ions on the membrane of a skeletal muscle fiber. Top—scheme showing the principle of the apparatus for stimulating single muscle fibers and recording the potentials from them. R_1 = 15 kΩ, R_2 = 50 kΩ, R_3 = 50 mΩ. The fall in voltage across the resistor R_1 (terminal I) was used as an indicator of the strength of the stimulating current; terminal II—output of cathode repeater. A) action potential of a muscle fiber in Ringer's solution of normal composition; B) the same after addition of 1 mmole $ZnCl_2$ to the solution; C) action of potential of a muscle fiber in Ringer's solution, the fiber is in a poor functional state; D) response of the same fiber after addition of 0.25 mmole $ZnCl_2$ to the Ringer's solution. The top curve corresponds to the zero line for the intracellular microelectrode, and the stimulating current applied to the fiber through the second microelectrode is also indicated on it (in tracings A and B). The bottom curve shows the potential recorded by the microelectrode.

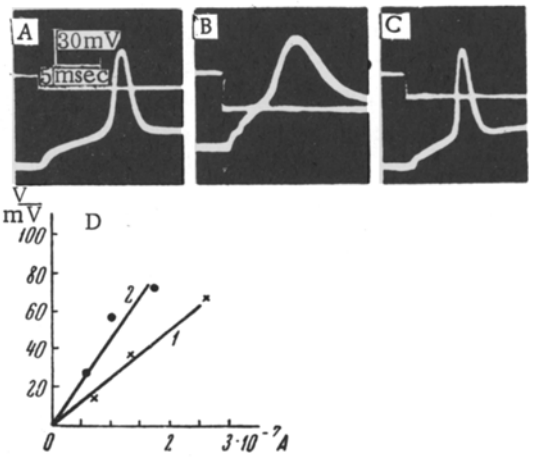

Fig. 2. Top: reversible character of the action of Zn ions. Bottom: volt-ampere characterisitcs of the membrane of a skeletal muscle fiber. A) action potential of a muscle fiber in Ringer's solution of normal composition; B) the same after addition of 1 mmole $ZnCl_2$ to the solution; C) restoration of the action potential after immersion of the muscle in a 0.1% solution of unithiol. Remainder of legend as in Fig. 1; D) volt-ampere characteristic of the membrane of a muscle fiber measured by means of hyperpolarizing pulses of current: 1) muscle in Ringer's solution; 2) the same muscle fiber in a solution of $ZnCl_2$ in a concentration of 1 mmole.

($P > 0.1$). Thirty fibers were tested in both solutions. As is clear from Fig. 1A and B, 1 mmole of $ZnCl_2$ lowered the RP from 90.4 ±6.15 to 73.05 ±3.8 mV (30 fibers) ($P < 0.01$) and increased the critical level of depolarization of the membrane from 47.5 ±2.15 to 37.3 ±2.1 mV (20 fibers; $P < 0.01$). In normal conditions the amplitude of the AP was 132.15 ±2.5 mV (25 fibers), but in the frogs in a poor functional condition the AP was lowered to 95.9 ±5.65 mV (15 fibers). The difference was significant ($P < 0.01$). The addition of Zn ions to the solution did not change the amplitude of the "normal" AP, but it substantially increased its duration on account of a prolongation of the ascending and, in particular, the descending phases. It is clear from Fig. 1C and D that in the fibers with a low amplitude of the AP (95.9 ±5.65 mV) Zn ions raised it to 127.5 ±2.9 mV (15 fibers; $P < 0.01$).

The input resistance of the membrane of the muscle fibers was increased under the influence of 1 mmole $ZnCl_2$ from 216 ±86 to 465 ±82.2 kΩ (15 fibers). The difference was significant ($0.01 < P < 0.02$). In the presence of $ZnCl_2$ in a concentration of 0.05-0.1 mmole, the input resistance of the fibers was unchanged. The effect of Zn ions was very stable and was not abolished even after repeated rinsing of the muscle in Ringer's solution. In contrast to this, replacement of the Ringer's solution containing Zn ions by a solution of unithiol or of cysteine in concentrations of 0.05-0.1% led to complete restoration of the original amplitude and duration of the AP (Fig. 2, A-C).

On the basis of modern views of the nature of the membrane potential [8-10], the lowering of the RP of the muscle fiber under the influence of 0.25-1.0 mmole $ZnCl_2$ may be explained either by an increase in the permeability

of the excitable membrane to Na ions (P_{Na}) or by a decrease in its permeability to K and Cl ions (P_K and P_{Cl}). Since the lowering of the RP under the influence of Zn ions was accompanied by a slight increase in the resistance of the muscle membrane (Fig. 2D), it may be considered that Zn^{++} ions in the concentrations mentioned lower the permeability of the membrane to K and Cl ions or to one of them. In concentrations less than 0.25 mmole, Zn ions evidently had no effect on the P_K and P_{Cl} of the resting membrane. A characteristic feature of the action of Zn^{++} ions on the muscle fiber was the marked increase in the duration of the ascending and, in particular, of the descending phase of the AP. Remembering that the gradient of the ascending phase of the AP is determined by the rate of increase of the P_{Na} of the membrane during its depolarization [8, 10], it may be concluded that Zn^{++} ions considerable depress the velocity constant [10] of this process.

The duration of the descending phase of the AP is known to depend on the speed of two processes: the process of inactivation of P_{Na} and the process leading to a delay in the increase in P_K. Before the question of which of these two processes is inhibited more by Zn ions can be answered, further investigations are required. Several studies have shown that ions of Ni, Co, Cd [10, 11, 13, 14] and, to a rather lesser degree, Zn [15] considerably prolong the descending phase of the AP of the medullated nerve fiber.

It has been concluded from recordings of the changes in the impedance of the membrane of the node of Ranvier during such a prolonged AP [11] and from the study of the effect of Ni and Cd ions on the resistance of the node to the depressant action of a cathode current, K ions, procaine, and mechanical injury [3], that Ni, Co, and Cd ions greatly inhibit the development of inactivation of the sodium permeability of the membrane. They have much less effect on the P_K system [2]. By analogy with this, it may be postulated that the effects of the action of Zn ions on the skeletal muscle fiber are also due mainly to their inhibitory influence on the development of the process of P_{Na} inactivation. This hypothesis is confirmed by the marked increase in the amplitude of the AP of skeletal muscle fibers when they are in a poor functional condition. Similar changes in the amplitude of the AP have also been observed in nodes of Ranvier showing functional changes under the influence of Ni and Cd ions, and they have been interpreted as the result of a decrease in the degree of inactivation of the sodium system of the membrane [3, 4, 11].

The similarity between the effect of these ions on nerve and muscle fibers is also confirmed by the fact that in both cases these effects are very stable and can be completely abolished only by the action of donors of sulfhydryl groups—cysteine or unithiol. The only difference between the effects obtained in nerve and muscle fibers is that the membrane of the nerve fibers is more sensitive to nickel ions while the membrane of the muscle fibers reacts to Zn ions but undergoes little change in its properties under the influence of nickel ions (the authors' preliminary data). The reason for these differences is not clear.

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